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(57) Abstract

Methods are provided for the therapy of immunodeficient, immunodepressed or hyperactive immune states and for the prevention and treatment of opportunistic infections in such states comprising administering to a subject a pharmaceutically acceptable composition comprising as an active ingredient the dipeptide L-Glu-L-Trp and/or its pharmaceutically acceptable salts.

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PHARMACEUTICAL DIPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF

This is a continuation-in-part of copending Serial No. 07/678,129, filed April 1, 1991.

The present invention is directed to dipeptide pharmaceutical compositions and uses thereof, in particular, uses thereof for treatment of immunodepressed states and of opportunistic infections in immunodepressed states.

BACKGROUND OF THE INVENTION

Several polypeptides found in the thymus gland have been implicated as playing roles in the development and maintenance of immunological competence in animals, including human beings. Some of these polypeptides have been shown to stimulate the maturation, differentiation and function of T-cells. For example, a heat-stable fraction isolated from calf thymus extracts, designated as Thymosin fraction 5, has been shown to reconstitute immune functions in thymic-deprived or immunodepressed individuals. Several peptides have been isolated from Thymosin fraction 5, such as Thymosin $alpha_1$ (28 amino acids, U.S. Patent No. 4,079,127), Thymosin $beta_4$ (44 amino acids, Low et al., PNAS, 78,1162-1166

(1981)), Thymosin $beta_8$ (39 amino acids, U.S. Patent No. 4,389,343) and Thymosin $beta_9$ (41 amino acids, U.S. Patent No. 4,389,343). However, practical administration of such polypeptides is expensive due to the relatively low yield and complexity of isolation and/or manufacture of such long chain polypeptides. Most importantly, in some cases, these polypeptides produce side reactions in patients.

The present invention is based in part on the discovery that a dipeptide, hereinafter referred to as Thymogen, exhibits a broad range of efficacy for prevention and treatment of opportunistic infections in immunodepressed states, and for therapeutically effective treatment of immunodeficient states. This is believed to be highly unexpected for such a relatively small compound to exhibit such a broad range of activity. Furthermore, we have not found any significant side effects from the use of the dipeptide according to the present invention. Due to its simple nature, the dipeptide is rather inexpensive to manufacture.

As used herein, the terms "immunomodulator" and "immunomodulating" encompass the activity of enhancing or restoring the subject's immune system, as evidenced by measurable blood parameters and/or the patient's improved ability to combat infection or disease, and the ability to heal tissue. Hence, immunomodulation encompasses improvement of the immune system due to an immunodeficient state (for example, caused by removal of the thymus), and/or an immunodepressed state (for example, caused by exposure to radiation). Furthermore, the present invention provides for modulation of the immune system by lowering blood parameters and other indicia of the immune state if these indicia are abnormally

elevated. The present invention encompasses the therapeutic method of treating the immunodeficient, immunodepressed or elevated immune state per se, thus providing prophylaxis against infection and disease, as well as a treatment of infection, disease or wound indirectly by enhancing the immune system.

It is therefore an object of the present invention to provide pharmaceutical compositions of the dipeptide Thymogen which have broad immunomodulating activity, as well as activity for other uses such as treatment of infections, disease and wounds (burns, frost bites, and the like), enhancement of metabolic processes, and many other uses.

It is an object of the present invention to provide therapeutic methods for treatment of immunodepressed and immunodeficient states.

It is yet another object of the present invention to provide methods for preventing and treating opportunistic infections in immunodeficient and immunodepressed states.

These and other objects will be apparent from the following description and appended claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides pharmaceutical preparations comprising the dipeptide L-Glu-L-Trp, using the normal convention wherein the first named amino acid is the amino terminus and the last named amino acid is the carboxyl terminus. The compositions according to the present invention may be formulated into any convenient formulation which allows for the active ingredient to be absorbed into

the blood stream. Intramuscular and intranasal forms of application are preferred. The preferred dosage rate of the active ingredient for intramuscular administration is about 50 to 100μg per dose for adults (for a 300 to 1000μg total treatment therapy); for infants up to 1 year old about 10μg per dose, for infants 1 to 3 years old about 10 to 20μg per dose; for infants 4 to 6 years old about 20 to 30μg per dose, for children 7 to 14 years old about 50μg per dose. All of the foregoing dosages are useful for a treatment of 3 to 10 days, depending upon the immunodeficiency level. The treatment may be repeated as needed, usually within 1 to 6 months.

For prophylactic uses against opportunistic infections in immunodeficient or immunodepressed patients, the intramuscular and/or intranasal single daily dose for adults may be from about 50 to 10μg, and for children about 10 to 50 μg per dose for treatment over 3 to 5 days.

For treatment of burns, frost bite, or other wounds, including chronic apical periodontitis, the dipeptide may be applied in about 100μg doses as a paste or other suitable medium.

For ophthalmology, such as for treatment of infectious eye diseases, the dipeptide may be applied in single daily dosages of about 10μg (over 4 to 10 days) or as installations into the conjunctival cavity at about 5μg twice daily over about 4 to 5 days.

The dipeptide may be utilized intramuscularly as an injection solution with the active ingredient in a therapeutically effective immunopotentiating amount of about .001 to .01% by weight. If presented in the

form of a tablet, capsule or suppository it is preferred that the active ingredient be present in an amount of about 0.1mg per tablet, suppository or capsule. If presented in such form, the capsule, suppository or tablet may also contain other conventional excipients and vehicles such as fillers, starch, glucose, etc.

The dipeptide may be obtained by conventional peptide synthesis, including the Merrifield solid state peptide synthesis technique. Typically an amino and side chain protected derivative of an activated ester of glutamic acid is reacted with protected L-tryptophan. After elimination of the protecting groups and conventional purification, such as by thin layer or GL chromatography, the peptide may be purified such as by, lyophilization, gel purification, and the like.

The purified dipeptide L-Glu-L-Trp, comprises a white powder (if lyophilized; otherwise, it is crystalline), soluble in water, DMF; insoluble in chloroform and ether. [alpha$^{22}_{D}$ = +12.6; C = 0.5 H_2O. R_f = 0.65 (butanol: acetic acid: water = 3:1:1). UV (275 ± 5nm, max). NMR (500MHz): 0.001mol/l of the peptide solution, Trp (3.17; 3.37; 4.57; 7.16; 7.24; 7.71; 7.49); Glu (1.90; 1.96; 2.21; 3.72).

The active dipeptide ingredient of the pharmaceutical preparations according to the present invention may be used as a free peptide or in the form of a water soluble pharmaceutically acceptable salt, such as a sodium, potassium, ammonium or zinc salt. It will be understood that the dipeptide may be administered with other active ingredients which independently